



(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
25.07.2001 Bulletin 2001/30

(51) Int Cl.7: **C12N 15/62, C12N 9/00,**
C12N 5/10, C12N 1/21,
C07K 14/435, C07K 14/035,
C07K 14/16, A01K 67/027,
A61K 38/43, A61K 47/48

(21) Application number: **00100351.6**

(22) Date of filing: **07.01.2000**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE
 Designated Extension States:
AL LT LV MK RO SI

(72) Inventor: **Schwenk, Frieder**
50739 Köln (DE)

(74) Representative:
Helbing, Jörg, Dr.Dipl.-Chem. et al
Patentanwälte
von Kreisler-Selting-Werner,
Deichmannhaus am Hauptbahnhof
50667 Köln (DE)

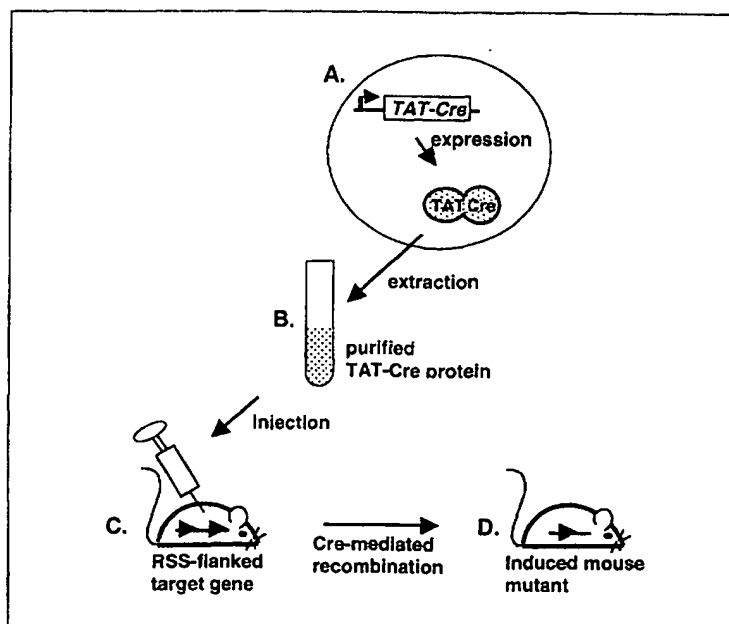
(71) Applicant: **ARTEMIS Pharmaceuticals GmbH**
51063 Köln (DE)

(54) **Transduction of recombinases for inducible gene targeting**

(57) The present invention provides the use of a fusion protein comprising a site-specific DNA recombinase domain and a protein transduction domain for pre-

paring an agent for inducing target gene alteration in a living organism, suitable fusion proteins and a method for the production of said fusion proteins.

Fig. 1



Description

[0001] The present invention provides the use of a fusion protein comprising a site-specific DNA recombinase domain and a protein transduction domain for preparing an agent for inducing target gene alteration in a living organism, suitable fusion proteins and a method for the production of said fusion proteins.

Background

[0002] For some years targeted mutagenesis in totipotent mouse embryonic stem (ES) cells has been used to inactivate genes, for which cloned sequences were available (Capecchi Trends in Genetics 5, 70 - 76 (1989)). Since ES cells can pass mutations induced in vitro to transgenic offspring in vivo, it is possible to analyze the consequences of gene disruption in the context of the entire organism. Thus, numerous mouse strains with functionally inactivated genes ("knock out mice") have been created by this technology and utilized to study the biological function of a variety of genes.

[0003] A refined method of targeted mutagenesis, referred to as conditional mutagenesis, employs a site-specific recombination system (e.g. Cre/loxP or Flp/rt - Sauer and Henderson, N. Proc. Natl. Acad. Sci. USA 85, 5166-5170 (1988); Senecoff et al., J. Mol. Biol., 201, 405 - 421 (1988)) which enables a temporally and/or spatially restricted alteration of target genes (Rajewsky et al, J. Clin. Invest., 98, 600 - 603 (1996)). The creation of conditional mouse mutants requires the generation of two mouse strains, i.e. the recombinase recognition strain and the recombinase expressing strain. The recombinase recognition strain is generated by homologous recombination in ES cells as described above except that the targeted exon(s) is (are) flanked by two recombinase recognition sequences (hereinafter "RRS"; e.g. loxP or rt). The type of recombination event mediated by the recombinase depends on the disposition of the RRS, with deletions, inversions, translocations and integrations being possible (Torres and Kühn, Oxford University Press, Oxford, New York (1997)). By placing the RRS into introns, an interference with gene expression before recombination can be avoided. The recombinase expressing strain contains a recombinase transgene (e.g. Cre, Flp) whose expression is either restricted to certain cells and tissues or is inducible by external agents. Crossing of the recombinase recognition strain with the recombinase expressing strain recombines the RRS-flanked exons from the doubly transgenic offspring in a prespecified temporally and/or spatially restricted manner. Thus, the method allows the temporal analysis of gene function in particular cells and tissues of otherwise widely expressed genes. Moreover, it enables the analysis of gene function in the adult organism by circumventing embryonic lethality which is frequently the consequence of gene mutation. For pharmaceutical research, aiming to validate the utility of genes and their products as targets for drug development, inducible mutations provide an excellent genetic tool. However, the current systems for inducible recombinase expression in transgenic animals suffer from a certain degree of leakiness in the absence of the inducer (Kühn et al., Science 269(5229):1427-9 (1995); Schwenk et al., Nucleic Acids Res; 26(6):1427-32 (1998)). Furthermore, the generation of conditional mutants is a time consuming and labor intensive procedure, since the recombinase recognition strain and the recombinase expressing strain have to be bred at least over two generations in order to obtain animals carrying both, the recombinase transgene and two copies of the RRS-flanked target gene sequence.

[0004] Protein domains that have the ability to cross cell membranes were identified in the Antennapedia protein from *Drosophila* (Vives et al., J Biol Chem, 272(25):16010-7 (1997)), VP22 from HSV (Elliott and O'Hare, Cell, 88(2): 223-33 (1997)) and TAT from HIV (Green and Loewenstein, Cell, 55(6):1179-88 (1988); Frankel and Pabo, Cell, 55(6): 1189-93 (1988)). Fusion of such domains to heterologous proteins conferred the ability to transduce into cultured cells (Fawell et al., Proc Natl Acad Sci U S A, 91(2):664-8 (1994); Elliott and O'Hare (1997), Phelan et al., Nature Biotech. 16; 440-443 (1998) and Dilber et al., Gene Ther., 6(1):12-21 (1999)). Dalby and Bennett showed that a fusion protein consisting of VP22 and functional Flp recombinase translocated between cells in culture (from COS-1 cells transfected with VP22-Flp to CHO cells carrying Flp recognition sites (FRT sites); see Dalby and Bennett, Invitrogen, Expressions 6.2, page 13 (1999)).

[0005] On the other hand, a recent report demonstrated that the β -galactosidase protein fused to the 11 amino acids transduction domain from the HIV TAT protein can infiltrate all tissues of living mice reaching every single cell (Schwarze et al., Science, 285(5433):1569-72 (1999)).

[0006] It was found that site-specific DNA recombinases can be translocated into cells of a living organism when fused to a protein transduction domain. Thus, whenever a gene mutation is desired, recombination is induced upon the injection of the appropriate site-specific recombinase fused to a transduction domain into such a living organism (provided, however, that said organism carries at least one appropriate RRS integrated in the genome).

[0007] The present invention thus provides

(1) the use of a fusion protein comprising

(a) a site-specific DNA recombinase domain and

(b) a protein transduction domain
for preparing an agent for inducing target gene alterations in a living organism, wherein said living organism carries at least one or more recognition sites for said site-specific DNA recombinase integrated in its genome;

(2) a method for inducing gene alterations in a living organism which comprises administering to said living organism a fusion protein comprising a site-specific DNA recombinase domain and a protein transduction domain as defined in (1) above, wherein said living organism carries at least one or more recognition sites for said site-specific DNA recombinase integrated in its genome;

(3) a fusion protein comprising

(a) a site-specific DNA recombinase domain and

(b) a protein transduction domain

provided that when the site-specific DNA recombinase domain is wild type F1p then the protein transduction domain is not the VP22 protein of HSV (i.e., the fusion protein is not identical to the fusion protein of Dalby and Bennett (1999));

(4) a DNA sequence coding for the fusion protein of (3) above;

(5) a vector comprising the DNA sequence as defined in (4) above;

(6) a host cell transformed with the vector of (5) above and/or comprising the DNA of (4) above;

(7) a method for producing the fusion protein of (1) above which comprises culturing the transformed host cell of (6) above and isolating the fusion protein; and

(8) an injectable composition comprising the fusion protein as defined in (1) or (3) above.

[0008] The invention is further illustrated by the appended Figure and is explained in detail below.

[0009] Figure 1: Generation of induced mouse mutants using purified fusion proteins.

A: Expression of the fusion protein consisting of the site-specific DNA recombinase (e.g. Cre) and the protein transduction domain (e.g. the HIV derived TAT peptide) in prokaryotic or eukaryotic cells.

B: Extraction and purification of the expressed fusion protein (e.g. as described in Nagahara et al., 1998).

C: Injection of the purified fusion protein into mice carrying the RRS-flanked target sequence.

D: Analysis of the pattern of induced target gene recombination and the resulting phenotype.

Triangle: RRS.

[0010] The expression "target sequences" according to the present invention means all kind of sequences which may be mutated (viz. deleted, translocated, integrated and/or inverted) by the action of the recombinase. The number of RRS in the target sequence depends on the kind of mutation to be performed by the recombinase. For most of the mutations (especially for deletions and inversions) two RRS are required which are flanking the sequence to be mutated (deleted or inverted). For some kinds of integrations only one RRS may be necessary within the target sequence.

[0011] The "living organisms" according to the present invention are multi-cell organisms and can be vertebrates such as mammals (e.g., rodents such as mice or rats) or non-mammals (e.g., fish) or can be invertebrates such as insects or worms. Most preferred living organisms are mice and fish.

[0012] The site-specific DNA recombinase domain within the fusion protein of the invention of the present application is preferably selected from a recombinase protein derived from Cre, F1p, ϕ C31 recombinase (Thorpe and Smith, Proc. Natl. Acad. Sci. USA, vol. 95, 5505-5510 (1998)) and R recombinase (Araki et al., J. Mol. Biol., 182, 191-203 (1985)). The preferred recombinases are Cre (e.g., the Cre variant of aa 15 to 357 of SEQ ID NO: 2 or aa 325-667 of SEQ ID NO: 6) and F1pe (i.e., the F1p variant of aa 15 to 437 of SEQ ID NO: 4 or aa 325 to 747 of SEQ ID NO: 8).

[0013] The protein transduction domain according to the present invention is preferably derived from the Antennapedia protein of Drosophila, from the VP22 protein of HSV or from the TAT protein of HIV. Preferably the protein transduction domain is derived from the TAT protein among which a TAT protein comprising the amino acid sequence shown in SEQ ID NO: 10 is most preferred.

[0014] The fusion of the two domains of the fusion protein can occur at any possible position, i.e., the protein transduction domain can be fused to the N- or C-terminal of the site-specific DNA recombinase or can be fused to active sites within the site-specific DNA recombinase. Preferably the protein transduction domain is fused to the N-terminal of the site-specific DNA recombinase domain.

[0015] The protein transduction domain can be fused to the site-specific DNA recombinase either through a direct chemical bond or through a linker molecule. Such linker molecule can be any bivalent chemical structure capable of linking the two domains. The preferred linker molecule according to the present invention is a short peptide, e.g., having 1 to 20, preferably 1 to 10, amino acid residues. Specifically preferred short peptides are essentially consisting of Gly, Ala and/or Leu.

[0016] The fusion protein of the invention of the present application may further comprise other functional sequences

such as secretion conferring signals, nuclear localization signals and/or signals conferring protein stabilization.

[0017] In case the fusion protein comprises a protein transduction domain derived from the TAT protein of HIV, the DNA sequence coding for said fusion protein preferably comprises the sequence

5

5' TAC GGC CGC AAG AAG CGC CGC CAA CGC CGC CGC 3'.

10

[0018] Such a preferred DNA sequence is for instance shown in SEQ ID NO: 11. In said sequence the 3' terminal codon ggc codes for the linker Gly. The DNA sequence of a suitable recombinase may be directly attached to said codon ggc.

[0019] The fusion protein can be obtained by the following steps:

15

1. Fusion of the recombinase coding region (e.g. encoding Cre: see amino acids 15 to 357 of SEQ ID NO: 2) with the sequence conferring protein translocation (e.g. the sequence encoding the TAT peptide YGRKKRRQRRR, SEQ ID NO: 10) using standard cloning protocols (Maniatis et al., Cold Spring Harbor Laboratory, New York (1989)) or chemical synthesis.

20

2. Generation of a construct for the expression of the fusion protein in prokaryotic or eukaryotic cells, e.g. in *E. coli* DH5a (Hanahan, J. Mol. Biol.; 166(4):557-80 (1983)) using the QIAexpress pQE vector (Qiagen, Hilden).

3. Expression of the above mentioned fusion protein in prokaryotic or eukaryotic cells, e.g. in *E. coli* DH5a (Hanahan, 1983)

4. Extraction and purification of the above mentioned fusion protein e.g. as described in Nagahara et al., Nat. Med., 4(12):1449-52 (1998).

25

[0020] Injection of the purified fusion protein into a living organism (e.g., a mouse) carrying a gene comprising the RRS-flanked target sequence (e.g., in an amount of 5 to 50 µg per g body weight). To demonstrate the feasibility of the invention, a reporter mouse strain is used harbouring a RRS-flanked cassette which, when deleted by the recombinase, allows the expression of a cellular marker protein, such as β-galactosidase (Thorey et al., Mol. Cell Biol., 18(10):6164 (1998)).

30

[0021] Analysis is achieved by determining the pattern of induced target gene recombination (e.g. through Southern blot analysis and X-Gal staining on tissue sections; Maniatis et al., 1989; Gossler and Zachgo, Joyner AL (Ed.), Oxford University Press, Oxford, New York (1993)).

[0022] The procedure's advantages over current technology are as follows:

35

(i) The absence of background recombination before administration of the fusion protein.

(ii) The reduction of time and resources which are necessary to combine the recombinase transgene and two copies of the RRS-flanked target gene by conventional breeding.

40

45

50

55

SEQUENCE LISTING

<110> ARTEMIS Pharmaceuticals GmbH

<120> Transduction of recombinases for inducible
gene targeting

<130> 992928ep/JH/ml

<140>

<141>

<160> 12

<170> PatentIn Ver. 2.1

<210> 1

<211> 1074

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: DNA sequence
coding for a fusion protein TAT-Cre

<220>

<221> CDS

<222> (1)..(1071)

<400> 1

atg ggc tac ggc cgc aag aag cgc cgc caa cgc cgc cgc ggc atg tcc	48
Met Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly Met Ser	
1 5 10 15	

aat tta ctg acc gta cac caa aat ttg cct gca tta ccg gtc gat gca	96
Asn Leu Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val Asp Ala	
20 25 30	

acg agt gat gag gtt cgc aag aac ctg atg gac atg ttc agg gat cgc	144
Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg Asp Arg	
35 40 45	

cag gcg ttt tct gag cat acc tgg aaa atg ctt ctg tcc gtt tgc cgg	192
Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val Cys Arg	
50 55 60	

tcg tgg gcg gca tgg tgc aag ttg aat aac cgg aaa tgg ttt ccc gca	240
Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe Pro Ala	
65 70 75 80	

gaa cct gaa gat gtt cgc gat tat ctt cta tat ctt cag gcg cgc ggt	288
Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala Arg Gly	
85 90 95	

ctg gca gta aaa act atc cag caa cat ttg ggc cag cta aac atg ctt	336
Leu Ala Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn Met Leu	
100 105 110	

EP 1 118 668 A1

	cat	cgt	cgg	tcc	ggg	ctg	cca	cga	cca	agt	gac	agc	aat	gct	gtt	tca	384
	His	Arg	Arg	Ser	Gly	Leu	Pro	Arg	Pro	Ser	Asp	Ser	Asn	Ala	Val	Ser	
			115					120					125				
5	ctg	gtt	atg	cgg	cgg	atc	cga	aaa	gaa	aac	gtt	gat	gcc	ggg	gaa	cgt	432
	Leu	Val	Met	Arg	Arg	Ile	Arg	Lys	Glu	Asn	Val	Asp	Ala	Gly	Glu	Arg	
			130				135					140					
10	gca	aaa	cag	gct	cta	gcg	ttc	gaa	cgc	act	gat	ttc	gac	cag	gtt	cgt	480
	Ala	Lys	Gln	Ala	Leu	Ala	Phe	Glu	Arg	Thr	Asp	Phe	Asp	Gln	Val	Arg	
	145					150					155					160	
15	tca	ctc	atg	gaa	aat	agc	gat	cgc	tgc	cag	gat	ata	cgt	aat	ctg	gca	528
	Ser	Leu	Met	Glu	Asn	Ser	Asp	Arg	Cys	Gln	Asp	Ile	Arg	Asn	Leu	Ala	
					165				170						175		
20	ttt	ctg	ggg	att	gct	tat	aac	acc	ctg	tta	cgt	ata	gcc	gaa	att	gcc	576
	Phe	Leu	Gly	Ile	Ala	Tyr	Asn	Thr	Leu	Leu	Arg	Ile	Ala	Glu	Ile	Ala	
				180					185					190			
25	agg	atc	agg	gtt	aaa	gat	atc	tca	cgt	act	gac	ggg	ggg	aga	atg	tta	624
	Arg	Ile	Arg	Val	Lys	Asp	Ile	Ser	Arg	Thr	Asp	Gly	Gly	Arg	Met	Leu	
				195				200					205				
30	atc	cat	att	ggc	aga	acg	aaa	acg	ctg	gtt	agc	acc	gca	ggg	gta	gag	672
	Ile	His	Ile	Gly	Arg	Thr	Lys	Thr	Leu	Val	Ser	Thr	Ala	Gly	Val	Glu	
			210				215					220					
35	aag	gca	ctt	agc	ctg	ggg	gta	act	aaa	ctg	gtc	gag	cga	tgg	att	tcc	720
	Lys	Ala	Leu	Ser	Leu	Gly	Val	Thr	Lys	Leu	Val	Glu	Arg	Trp	Ile	Ser	
	225					230					235					240	
40	gtc	tct	ggg	gta	gct	gat	gat	ccg	aat	aac	tac	ctg	ttt	tgc	cgg	gtc	768
	Val	Ser	Gly	Val	Ala	Asp	Asp	Pro	Asn	Asn	Tyr	Leu	Phe	Cys	Arg	Val	
					245					250					255		
45	aga	aaa	aat	ggg	gtt	gcc	gcg	cca	tct	gcc	acc	agc	cag	cta	tca	act	816
	Arg	Lys	Asn	Gly	Val	Ala	Ala	Pro	Ser	Ala	Thr	Ser	Gln	Leu	Ser	Thr	
				260					265					270			
50	cgc	gcc	ctg	gaa	ggg	att	ttt	gaa	gca	act	cat	cga	ttg	att	tac	ggc	864
	Arg	Ala	Leu	Glu	Gly	Ile	Phe	Glu	Ala	Thr	His	Arg	Leu	Ile	Tyr	Gly	
			275					280					285				
55	gct	aag	gat	gac	tct	ggg	cag	aga	tac	ctg	gcc	tgg	tct	gga	cac	agt	912
	Ala	Lys	Asp	Asp	Ser	Gly	Gln	Arg	Tyr	Leu	Ala	Trp	Ser	Gly	His	Ser	
			290				295					300					
60	gcc	cgt	gtc	gga	gcc	gcg	cga	gat	atg	gcc	cgc	gct	gga	gtt	tca	ata	960
	Ala	Arg	Val	Gly	Ala	Ala	Arg	Asp	Met	Ala	Arg	Ala	Gly	Val	Ser	Ile	
	305					310					315					320	
65	ccg	gag	atc	atg	caa	gct	ggg	ggc	tgg	acc	aat	gta	aat	att	gtc	atg	1008
	Pro	Glu	Ile	Met	Gln	Ala	Gly	Gly	Trp	Thr	Asn	Val	Asn	Ile	Val	Met	
					325					330					335		
70	aac	tat	atc	cgt	aac	ctg	gat	agt	gaa	aca	ggg	gca	atg	gtg	cgc	ctg	1056
	Asn	Tyr	Ile	Arg	Asn	Leu	Asp	Ser	Glu	Thr	Gly	Ala	Met	Val	Arg	Leu	
				340					345					350			

ctg gaa gat ggc gat tag
 Leu Glu Asp Gly Asp
 355

5

<210> 2
 <211> 357
 <212> PRT
 <213> Artificial Sequence
 <223> Description of Artificial Sequence: DNA sequence
 coding for a fusion protein TAT-Cre

10

<400> 2
 Met Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly Met Ser
 1 5 10 15

15

Asn Leu Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val Asp Ala
 20 25 30

20

Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg Asp Arg
 35 40 45

Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val Cys Arg
 50 55 60

25

Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe Pro Ala
 65 70 75 80

Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala Arg Gly
 85 90 95

30

Leu Ala Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn Met Leu
 100 105 110

His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala Val Ser
 115 120 125

35

Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly Glu Arg
 130 135 140

Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln Val Arg
 145 150 155 160

40

Ser Leu Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn Leu Ala
 165 170 175

Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu Ile Ala
 180 185 190

45

Arg Ile Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg Met Leu
 195 200 205

Ile His Ile Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly Val Glu
 210 215 220

50

Lys Ala Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp Ile Ser
 225 230 235 240

Val Ser Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys Arg Val
 245 250 255

55

EP 1 118 668 A1

Arg Lys Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu Ser Thr
 260 265 270
 5 Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile Tyr Gly
 275 280 285
 Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly His Ser
 290 295 300
 10 Ala Arg Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val Ser Ile
 305 310 315 320
 Pro Glu Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile Val Met
 325 330 335
 15 Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val Arg Leu
 340 345 350
 Leu Glu Asp Gly Asp
 355
 20
 <210> 3
 <211> 1317
 <212> DNA
 25 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: DNA sequence
 coding for a fusion protein TAT-Flpe
 30
 <220>
 <221> CDS
 <222> (1)..(1311)
 35
 <400> 3
 atg ggc tac ggc cgc aag aag cgc cgc caa cgc cgc cgc ggc atg agt 48
 Met Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly Met Ser
 1 5 10 15
 caa ttt gat ata tta tgt aaa aca cca cct aag gtc ctg gtt cgt cag 96
 40 Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro Lys Val Leu Val Arg Gln
 20 25 30
 ttt gtg gaa agg ttt gaa aga cct tca ggg gaa aaa ata gca tca tgt 144
 Phe Val Glu Arg Phe Glu Arg Pro Ser Gly Glu Lys Ile Ala Ser Cys
 35 40 45
 45 gct gct gaa cta acc tat tta tgt tgg atg att act cat aac gga aca 192
 Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met Ile Thr His Asn Gly Thr
 50 55 60
 50 gca atc aag aga gcc aca ttc atg agc tat aat act atc ata agc aat 240
 Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr Asn Thr Ile Ile Ser Asn
 65 70 75 80
 tcg ctg agt ttc gat att gtc aac aaa tca ctc cag ttt aaa tac aag 288
 55 Ser Leu Ser Phe Asp Ile Val Asn Lys Ser Leu Gln Phe Lys Tyr Lys
 85 90 95

EP 1 118 668 A1

5	acg caa aaa gca aca att ctg gaa gcc tca tta aag aaa tta att cct Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser Leu Lys Lys Leu Ile Pro	336
	100 105 110	
	gct tgg gaa ttt aca att att cct tac aat gga caa aaa cat caa tct Ala Trp Glu Phe Thr Ile Ile Pro Tyr Asn Gly Gln Lys His Gln Ser	384
	115 120 125	
10	gat atc act gat att gta agt agt ttg caa tta cag ttc gaa tca tcg Asp Ile Thr Asp Ile Val Ser Ser Leu Gln Leu Gln Phe Glu Ser Ser	432
	130 135 140	
15	gaa gaa gca gat aag gga aat agc cac agt aaa aaa atg ctt aaa gca Glu Glu Ala Asp Lys Gly Asn Ser His Ser Lys Lys Met Leu Lys Ala	480
	145 150 155 160	
20	ctt cta agt gag ggt gaa agc atc tgg gag atc act gag aaa ata cta Leu Leu Ser Glu Gly Glu Ser Ile Trp Glu Ile Thr Glu Lys Ile Leu	528
	165 170 175	
	aat tcg ttt gag tat acc tcg aga ttt aca aaa aca aaa act tta tac Asn Ser Phe Glu Tyr Thr Ser Arg Phe Thr Lys Thr Lys Thr Leu Tyr	576
	180 185 190	
25	caa ttc ctc ttc cta gct act ttc atc aat tgt gga aga ttc agc gat Gln Phe Leu Phe Leu Ala Thr Phe Ile Asn Cys Gly Arg Phe Ser Asp	624
	195 200 205	
30	att aag aac gtt gat ccg aaa tca ttt aaa tta gtc caa aat aag tat Ile Lys Asn Val Asp Pro Lys Ser Phe Lys Leu Val Gln Asn Lys Tyr	672
	210 215 220	
	ctg gga gta ata atc cag tgt tta gtg aca gag aca aag aca agc gtt Leu Gly Val Ile Ile Gln Cys Leu Val Thr Glu Thr Lys Thr Ser Val	720
	225 230 235 240	
35	agt agg cac ata tac ttc ttt agc gca agg ggt agg atc gat cca ctt Ser Arg His Ile Tyr Phe Phe Ser Ala Arg Gly Arg Ile Asp Pro Leu	768
	245 250 255	
40	gta tat ttg gat gaa ttt ttg agg aat tct gaa cca gtc cta aaa cga Val Tyr Leu Asp Glu Phe Leu Arg Asn Ser Glu Pro Val Leu Lys Arg	816
	260 265 270	
45	gta aat agg acc ggc aat tct tca agc aac aaa cag gaa tac caa tta Val Asn Arg Thr Gly Asn Ser Ser Ser Asn Lys Gln Glu Tyr Gln Leu	864
	275 280 285	
	tta aaa gat aac tta gtc aga tcg tac aac aag gct ttg aag aaa aat Leu Lys Asp Asn Leu Val Arg Ser Tyr Asn Lys Ala Leu Lys Lys Asn	912
	290 295 300	
50	gcg cct tat cca atc ttt gct ata aag aat ggc cca aaa tct cac att Ala Pro Tyr Pro Ile Phe Ala Ile Lys Asn Gly Pro Lys Ser His Ile	960
	305 310 315 320	
55	gga aga cat ttg atg acc tca ttt ctg tca atg aag ggc cta acg gag Gly Arg His Leu Met Thr Ser Phe Leu Ser Met Lys Gly Leu Thr Glu	1008
	325 330 335	

EP 1 118 668 A1

5 ttg act aat gtt gtg gga aat tgg agc gat aag cgt gct tct gcc gtg 1056
 Leu Thr Asn Val Val Gly Asn Trp Ser Asp Lys Arg Ala Ser Ala Val
 340 345 350

 gcc agg aca acg tat act cat cag ata aca gca ata cct gat cac tac 1104
 Ala Arg Thr Thr Tyr Thr His Gln Ile Thr Ala Ile Pro Asp His Tyr
 355 360 365

10 ttc gca cta gtt tct cgg tac tat gca tat gat cca ata tca aag gaa 1152
 Phe Ala Leu Val Ser Arg Tyr Tyr Ala Tyr Asp Pro Ile Ser Lys Glu
 370 375 380

15 atg ata gca ttg aag gat gag act aat cca att gag gag tgg cag cat 1200
 Met Ile Ala Leu Lys Asp Glu Thr Asn Pro Ile Glu Glu Trp Gln His
 385 390 395 400

20 ata gaa cag cta aag ggt agt gct gaa gga agc ata cga tac ccc gca 1248
 Ile Glu Gln Leu Lys Gly Ser Ala Glu Gly Ser Ile Arg Tyr Pro Ala
 405 410 415

25 tgg aat ggg ata ata tca cag gag gta cta gac tac ctt tca tcc tac 1296
 Trp Asn Gly Ile Ile Ser Gln Glu Val Leu Asp Tyr Leu Ser Ser Tyr
 420 425 430

 ata aat aga cgc ata taatga 1317
 Ile Asn Arg Arg Ile
 435

30 <210> 4
 <211> 437
 <212> PRT
 <213> Artificial Sequence
 <223> Description of Artificial Sequence: DNA sequence
 coding for a fusion protein TAT-Flpe

35 <400> 4
 Met Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly Met Ser
 1 5 10 15

40 Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro Lys Val Leu Val Arg Gln
 20 25 30

 Phe Val Glu Arg Phe Glu Arg Pro Ser Gly Glu Lys Ile Ala Ser Cys
 35 40 45

45 Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met Ile Thr His Asn Gly Thr
 50 55 60

50 Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr Asn Thr Ile Ile Ser Asn
 65 70 75 80

 Ser Leu Ser Phe Asp Ile Val Asn Lys Ser Leu Gln Phe Lys Tyr Lys
 85 90 95

55 Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser Leu Lys Lys Leu Ile Pro
 100 105 110

EP 1 118 668 A1

	Ala	Trp	Glu	Phe	Thr	Ile	Ile	Pro	Tyr	Asn	Gly	Gln	Lys	His	Gln	Ser	
			115					120					125				
5	Asp	Ile	Thr	Asp	Ile	Val	Ser	Ser	Leu	Gln	Leu	Gln	Phe	Glu	Ser	Ser	
		130					135					140					
	Glu	Glu	Ala	Asp	Lys	Gly	Asn	Ser	His	Ser	Lys	Lys	Met	Leu	Lys	Ala	
	145					150					155					160	
10	Leu	Leu	Ser	Glu	Gly	Glu	Ser	Ile	Trp	Glu	Ile	Thr	Glu	Lys	Ile	Leu	
					165					170					175		
	Asn	Ser	Phe	Glu	Tyr	Thr	Ser	Arg	Phe	Thr	Lys	Thr	Lys	Thr	Leu	Tyr	
15				180					185						190		
	Gln	Phe	Leu	Phe	Leu	Ala	Thr	Phe	Ile	Asn	Cys	Gly	Arg	Phe	Ser	Asp	
			195					200					205				
	Ile	Lys	Asn	Val	Asp	Pro	Lys	Ser	Phe	Lys	Leu	Val	Gln	Asn	Lys	Tyr	
20		210					215					220					
	Leu	Gly	Val	Ile	Ile	Gln	Cys	Leu	Val	Thr	Glu	Thr	Lys	Thr	Ser	Val	
	225					230					235					240	
	Ser	Arg	His	Ile	Tyr	Phe	Phe	Ser	Ala	Arg	Gly	Arg	Ile	Asp	Pro	Leu	
25				245					250						255		
	Val	Tyr	Leu	Asp	Glu	Phe	Leu	Arg	Asn	Ser	Glu	Pro	Val	Leu	Lys	Arg	
				260					265					270			
30	Val	Asn	Arg	Thr	Gly	Asn	Ser	Ser	Ser	Asn	Lys	Gln	Glu	Tyr	Gln	Leu	
			275					280					285				
	Leu	Lys	Asp	Asn	Leu	Val	Arg	Ser	Tyr	Asn	Lys	Ala	Leu	Lys	Lys	Asn	
		290					295					300					
35	Ala	Pro	Tyr	Pro	Ile	Phe	Ala	Ile	Lys	Asn	Gly	Pro	Lys	Ser	His	Ile	
	305					310					315					320	
	Gly	Arg	His	Leu	Met	Thr	Ser	Phe	Leu	Ser	Met	Lys	Gly	Leu	Thr	Glu	
					325					330					335		
40	Leu	Thr	Asn	Val	Val	Gly	Asn	Trp	Ser	Asp	Lys	Arg	Ala	Ser	Ala	Val	
				340					345					350			
	Ala	Arg	Thr	Thr	Tyr	Thr	His	Gln	Ile	Thr	Ala	Ile	Pro	Asp	His	Tyr	
45			355					360					365				
	Phe	Ala	Leu	Val	Ser	Arg	Tyr	Tyr	Ala	Tyr	Asp	Pro	Ile	Ser	Lys	Glu	
		370					375					380					
	Met	Ile	Ala	Leu	Lys	Asp	Glu	Thr	Asn	Pro	Ile	Glu	Glu	Trp	Gln	His	
50		385				390					395					400	
	Ile	Glu	Gln	Leu	Lys	Gly	Ser	Ala	Glu	Gly	Ser	Ile	Arg	Tyr	Pro	Ala	
				405						410					415		
	Trp	Asn	Gly	Ile	Ile	Ser	Gln	Glu	Val	Leu	Asp	Tyr	Leu	Ser	Ser	Tyr	
55				420					425					430			

EP 1 118 668 A1

Ile Asn Arg Arg Ile
435

5

<210> 5
<211> 2004
<212> DNA
<213> Artificial Sequence

10

<220>
<223> Description of Artificial Sequence: DNA sequence
coding for a fusion protein VP22-Cre

15

<220>
<221> CDS
<222> (1)..(2001)

20

<400> 5
atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc 48
Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg
1 5 10 15

25

gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt 96
Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser
20 25 30

30

ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc 144
Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg
35 40 45

tcg cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat 192
Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp
50 55 60

35

tat gcc ctc tac ggg ggc tcg tct tcc gaa gac gac gaa cac ccg gag 240
Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu
65 70 75 80

40

gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg 288
Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro
85 90 95

ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga 336
Gly Pro Ala Arg Ala Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly
100 105 110

45

cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg 384
Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala
115 120 125

50

act aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa 432
Thr Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys
130 135 140

55

tcg gcc cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg 480
Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr
145 150 155 160

EP 1 118 668 A1

	gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg	528
	Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu	
	165 170 175	
5	cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg	576
	His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg	
	180 185 190	
10	gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg	624
	Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu	
	195 200 205	
15	gcg gcc atg cat gcc cgg atg gcg gcg gtc cag ctc tgg gac atg tcg	672
	Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser	
	210 215 220	
20	cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc	720
	Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr	
	225 230 235 240	
25	atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac	768
	Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn	
	245 250 255	
30	gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg	816
	Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala	
	260 265 270	
35	act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc	864
	Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala	
	275 280 285	
40	cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag ggt acc gag	912
	Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu	
	290 295 300	
45	ctc gga tcc act agt cca gtg tgg tgg aat tct gca gat atc cag cac	960
	Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His	
	305 310 315 320	
50	agt ggc ggc cgc atg tcc aat tta ctg acc gta cac caa aat ttg cct	1008
	Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro	
	325 330 335	
55	gca tta ccg gtc gat gca acg agt gat gag gtt cgc aag aac ctg atg	1056
	Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met	
	340 345 350	
60	gac atg ttc agg gat cgc cag gcg ttt tct gag cat acc tgg aaa atg	1104
	Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met	
	355 360 365	
65	ctt ctg tcc gtt tgc cgg tcg tgg gcg gca tgg tgc aag ttg aat aac	1152
	Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn	
	370 375 380	
70	cgg aaa tgg ttt ccc gca gaa cct gaa gat gtt cgc gat tat ctt cta	1200
	Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu	
	385 390 395 400	

EP 1 118 668 A1

5	tat ctt cag gcg cgc ggt ctg gca gta aaa act atc cag caa cat ttg Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu	1248
	405 410 415	
	ggc cag cta aac atg ctt cat cgt cgg tcc ggg ctg cca cga cca agt Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser	1296
	420 425 430	
10	gac agc aat gct gtt tca ctg gtt atg cgg cgg atc cga aaa gaa aac Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn	1344
	435 440 445	
15	gtt gat gcc ggt gaa cgt gca aaa cag gct cta gcg ttc gaa cgc act Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr	1392
	450 455 460	
20	gat ttc gac cag gtt cgt tca ctc atg gaa aat agc gat cgc tgc cag Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln	1440
	465 470 475 480	
25	gat ata cgt aat ctg gca ttt ctg ggg att gct tat aac acc ctg tta Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu	1488
	485 490 495	
	cgt ata gcc gaa att gcc agg atc agg gtt aaa gat atc tca cgt act Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr	1536
	500 505 510	
30	gac ggt ggg aga atg tta atc cat att ggc aga acg aaa acg ctg gtt Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val	1584
	515 520 525	
35	agc acc gca ggt gta gag aag gca ctt agc ctg ggg gta act aaa ctg Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu	1632
	530 535 540	
40	gtc gag cga tgg att tcc gtc tct ggt gta gct gat gat ccg aat aac Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn	1680
	545 550 555 560	
	tac ctg ttt tgc cgg gtc aga aaa aat ggt gtt gcc gcg cca tct gcc Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala	1728
	565 570 575	
45	acc agc cag cta tca act cgc gcc ctg gaa ggg att ttt gaa gca act Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr	1776
	580 585 590	
50	cat cga ttg att tac ggc gct aag gat gac tct ggt cag aga tac ctg His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu	1824
	595 600 605	
	gcc tgg tct gga cac agt gcc cgt gtc gga gcc gcg cga gat atg gcc Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Arg Asp Met Ala	1872
	610 615 620	
55	cgc gct gga gtt tca ata ccg gag atc atg caa gct ggt ggc tgg acc	1920

EP 1 118 668 A1

	Arg	Ala	Gly	Val	Ser	Ile	Pro	Glu	Ile	Met	Gln	Ala	Gly	Gly	Trp	Thr	
	625					630					635					640	
5	aat	gta	aat	att	gtc	atg	aac	tat	atc	cgt	aac	ctg	gat	agt	gaa	aca	1968
	Asn	Val	Asn	Ile	Val	Met	Asn	Tyr	Ile	Arg	Asn	Leu	Asp	Ser	Glu	Thr	
					645					650					655		
	ggg	gca	atg	gtg	cgc	ctg	ctg	gaa	gat	ggc	gat	tag					2004
10	Gly	Ala	Met	Val	Arg	Leu	Leu	Glu	Asp	Gly	Asp						
				660					665								
	<210> 6																
	<211> 667																
15	<212> PRT																
	<213> Artificial Sequence																
	<223> Description of Artificial Sequence: DNA sequence																
	coding for a fusion protein VP22-Cre																
20	<400> 6																
	Met	Thr	Ser	Arg	Arg	Ser	Val	Lys	Ser	Gly	Pro	Arg	Glu	Val	Pro	Arg	
	1				5					10					15		
25	Asp	Glu	Tyr	Glu	Asp	Leu	Tyr	Tyr	Thr	Pro	Ser	Ser	Gly	Met	Ala	Ser	
				20					25					30			
	Pro	Asp	Ser	Pro	Pro	Asp	Thr	Ser	Arg	Arg	Gly	Ala	Leu	Gln	Thr	Arg	
			35				40						45				
30	Ser	Arg	Gln	Arg	Gly	Glu	Val	Arg	Phe	Val	Gln	Tyr	Asp	Glu	Ser	Asp	
	50					55						60					
	Tyr	Ala	Leu	Tyr	Gly	Gly	Ser	Ser	Ser	Glu	Asp	Asp	Glu	His	Pro	Glu	
	65				70					75					80		
35	Val	Pro	Arg	Thr	Arg	Arg	Pro	Val	Ser	Gly	Ala	Val	Leu	Ser	Gly	Pro	
				85						90					95		
	Gly	Pro	Ala	Arg	Ala	Pro	Pro	Pro	Pro	Ala	Gly	Ser	Gly	Gly	Ala	Gly	
				100					105					110			
40	Arg	Thr	Pro	Thr	Thr	Ala	Pro	Arg	Ala	Pro	Arg	Thr	Gln	Arg	Val	Ala	
			115				120						125				
	Thr	Lys	Ala	Pro	Ala	Ala	Pro	Ala	Ala	Glu	Thr	Thr	Arg	Gly	Arg	Lys	
45		130					135					140					
	Ser	Ala	Gln	Pro	Glu	Ser	Ala	Ala	Leu	Pro	Asp	Ala	Pro	Ala	Ser	Thr	
	145				150					155					160		
	Ala	Pro	Thr	Arg	Ser	Lys	Thr	Pro	Ala	Gln	Gly	Leu	Ala	Arg	Lys	Leu	
50				165						170					175		
	His	Phe	Ser	Thr	Ala	Pro	Pro	Asn	Pro	Asp	Ala	Pro	Trp	Thr	Pro	Arg	
				180				185						190			
55	Val	Ala	Gly	Phe	Asn	Lys	Arg	Val	Phe	Cys	Ala	Ala	Val	Gly	Arg	Leu	
			195				200						205				

EP 1 118 668 A1

	Ala	Ala	Met	His	Ala	Arg	Met	Ala	Ala	Val	Gln	Leu	Trp	Asp	Met	Ser	
	210						215					220					
5	Arg	Pro	Arg	Thr	Asp	Glu	Asp	Leu	Asn	Glu	Leu	Leu	Gly	Ile	Thr	Thr	
	225					230					235					240	
	Ile	Arg	Val	Thr	Val	Cys	Glu	Gly	Lys	Asn	Leu	Leu	Gln	Arg	Ala	Asn	
					245					250					255		
10	Glu	Leu	Val	Asn	Pro	Asp	Val	Val	Gln	Asp	Val	Asp	Ala	Ala	Thr	Ala	
				260					265					270			
	Thr	Arg	Gly	Arg	Ser	Ala	Ala	Ser	Arg	Pro	Thr	Glu	Arg	Pro	Arg	Ala	
			275					280					285				
15	Pro	Ala	Arg	Ser	Ala	Ser	Arg	Pro	Arg	Arg	Pro	Val	Glu	Gly	Thr	Glu	
		290					295					300					
	Leu	Gly	Ser	Thr	Ser	Pro	Val	Trp	Trp	Asn	Ser	Ala	Asp	Ile	Gln	His	
20	305					310					315					320	
	Ser	Gly	Gly	Arg	Met	Ser	Asn	Leu	Leu	Thr	Val	His	Gln	Asn	Leu	Pro	
					325					330					335		
25	Ala	Leu	Pro	Val	Asp	Ala	Thr	Ser	Asp	Glu	Val	Arg	Lys	Asn	Leu	Met	
				340					345					350			
	Asp	Met	Phe	Arg	Asp	Arg	Gln	Ala	Phe	Ser	Glu	His	Thr	Trp	Lys	Met	
			355					360					365				
30	Leu	Leu	Ser	Val	Cys	Arg	Ser	Trp	Ala	Ala	Trp	Cys	Lys	Leu	Asn	Asn	
		370					375					380					
	Arg	Lys	Trp	Phe	Pro	Ala	Glu	Pro	Glu	Asp	Val	Arg	Asp	Tyr	Leu	Leu	
	385					390					395					400	
35	Tyr	Leu	Gln	Ala	Arg	Gly	Leu	Ala	Val	Lys	Thr	Ile	Gln	Gln	His	Leu	
					405					410					415		
	Gly	Gln	Leu	Asn	Met	Leu	His	Arg	Arg	Ser	Gly	Leu	Pro	Arg	Pro	Ser	
40				420					425					430			
	Asp	Ser	Asn	Ala	Val	Ser	Leu	Val	Met	Arg	Arg	Ile	Arg	Lys	Glu	Asn	
			435					440					445				
45	Val	Asp	Ala	Gly	Glu	Arg	Ala	Lys	Gln	Ala	Leu	Ala	Phe	Glu	Arg	Thr	
		450					455					460					
	Asp	Phe	Asp	Gln	Val	Arg	Ser	Leu	Met	Glu	Asn	Ser	Asp	Arg	Cys	Gln	
	465					470					475					480	
50	Asp	Ile	Arg	Asn	Leu	Ala	Phe	Leu	Gly	Ile	Ala	Tyr	Asn	Thr	Leu	Leu	
					485					490					495		
	Arg	Ile	Ala	Glu	Ile	Ala	Arg	Ile	Arg	Val	Lys	Asp	Ile	Ser	Arg	Thr	
				500					505					510			
55	Asp	Gly	Gly	Arg	Met	Leu	Ile	His	Ile	Gly	Arg	Thr	Lys	Thr	Leu	Val	

EP 1 118 668 A1

	515		520		525	
5	Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu	530	535	540		
	Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn	545	550	555	560	
10	Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala	565	570	575		
	Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr	580	585	590		
15	His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu	595	600	605		
	Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala	610	615	620		
20	Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr	625	630	635	640	
	Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr	645	650	655		
25	Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp	660	665			
30	<210> 7					
	<211> 2004					
	<212> DNA					
	<213> Artificial Sequence					
35	<220>					
	<223> Description of Artificial Sequence: DNA sequence					
	coding for a fusion protein VP22-Flpe					
40	<220>					
	<221> CDS					
	<222> (1)..(1998)					
45	<400> 7					
	atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc				48	
	Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg	1	5	10	15	
50	gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt				96	
	Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser	20	25	30		
55	ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc				144	
	Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg	35	40	45		
55	tcg cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat				192	
	Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp	50	55	60		

EP 1 118 668 A1

	tat gcc ctc tac ggg ggc tcg tct tcc gaa gac gac gaa cac ccg gag	240
	Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu	
	65 70 75 80	
5	gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg	288
	Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro	
	85 90 95	
10	ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga	336
	Gly Pro Ala Arg Ala Pro Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly	
	100 105 110	
15	cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg	384
	Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala	
	115 120 125	
	act aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa	432
	Thr Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys	
	130 135 140	
20	tcg gcc cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg	480
	Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr	
	145 150 155 160	
25	gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg	528
	Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu	
	165 170 175	
30	cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg	576
	His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg	
	180 185 190	
	gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg	624
	Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu	
	195 200 205	
35	gcg gcc atg cat gcc cgg atg gcg gcg gtc cag ctc tgg gac atg tcg	672
	Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser	
	210 215 220	
40	cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc	720
	Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr	
	225 230 235 240	
	atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac	768
	Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn	
	245 250 255	
45	gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg	816
	Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala	
	260 265 270	
50	act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc	864
	Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala	
	275 280 285	
55	cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag ggt acc gag	912
	Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu	
	290 295 300	

EP 1 118 668 A1

	ctc gga tcc act agt cca gtg tgg tgg aat tct gca gat atc cag cac	960
	Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His	
	305 310 315 320	
5	agt ggc ggc cgc atg tcc aat tta ctg acc gta cac caa aat ttg cct	1008
	Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro	
	325 330 335	
10	gca tta ccg gtc gat gca acg agt gat gag gtt cgc aag aac ctg atg	1056
	Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met	
	340 345 350	
15	gac atg ttc agg gat cgc cag gcg ttt tct gag cat acc tgg aaa atg	1104
	Asp Met Phe Arg Asp Arg Gln Phe Ser Glu His Thr Trp Lys Met	
	355 360 365	
	ctt ctg tcc gtt tgc cgg tcg tgg gcg gca tgg tgc aag ttg aat aac	1152
	Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn	
	370 375 380	
20	cgg aaa tgg ttt ccc gca gaa cct gaa gat gtt cgc gat tat ctt cta	1200
	Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu	
	385 390 395 400	
25	tat ctt cag gcg cgc ggt ctg gca gta aaa act atc cag caa cat ttg	1248
	Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu	
	405 410 415	
30	ggc cag cta aac atg ctt cat cgt cgg tcc ggg ctg cca cga cca agt	1296
	Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser	
	420 425 430	
	gac agc aat gct gtt tca ctg gtt atg cgg cgg atc cga aaa gaa aac	1344
	Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn	
	435 440 445	
35	gtt gat gcc ggt gaa cgt gca aaa cag gct cta gcg ttc gaa cgc act	1392
	Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr	
	450 455 460	
40	gat ttc gac cag gtt cgt tca ctc atg gaa aat agc gat cgc tgc cag	1440
	Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln	
	465 470 475 480	
45	gat ata cgt aat ctg gca ttt ctg ggg att gct tat aac acc ctg tta	1488
	Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu	
	485 490 495	
	cgt ata gcc gaa att gcc agg atc agg gtt aaa gat atc tca cgt act	1536
	Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr	
	500 505 510	
50	gac ggt ggg aga atg tta atc cat att ggc aga acg aaa acg ctg gtt	1584
	Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val	
	515 520 525	
55	agc acc gca ggt gta gag aag gca ctt agc ctg ggg gta act aaa ctg	1632
	Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu	
	530 535 540	

EP 1 118 668 A1

5 gtc gag cga tgg att tcc gtc tct ggt gta gct gat gat ccg aat aac 1680
Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn 560
545 550 555

10 tac ctg ttt tgc cgg gtc aga aaa aat ggt gtt gcc gcg cca tct gcc 1728
Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala 575
565 570

15 acc agc cag cta tca act cgc gcc ctg gaa ggg att ttt gaa gca act 1776
Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr 590
580 585

20 cat cga ttg att tac ggc gct aag gat gac tct ggt cag aga tac ctg 1824
His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu 605
595 600

25 gcc tgg tct gga cac agt gcc cgt gtc gga gcc gcg cga gat atg gcc 1872
Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala 620
610 615

30 cgc gct gga gtt tca ata ccg gag atc atg caa gct ggt ggc tgg acc 1920
Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr 640
625 630 635

35 aat gta aat att gtc atg aac tat atc cgt aac ctg gat agt gaa aca 1968
Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr 655
645 650

40 ggg gca atg gtg cgc ctg ctg gaa gat ggc gattag 2004
Gly Ala Met Val Arg Leu Leu Glu Asp Gly 665
660

45 <210> 8
<211> 666
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: DNA sequence
coding for a fusion protein VP22-Flpe

50 <400> 8
Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg 15
1 5 10

55 Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser 30
20 25 30

Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg 45
35 40 45

60 Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp 60
50 55 60

65 Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu 80
65 70 75 80

70 Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro

EP 1 118 668 A1

	85	90	95
5	Gly Pro Ala Arg Ala Pro Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly 100 105 110		
	Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala 115 120 125		
10	Thr Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys 130 135 140		
	Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr 145 150 155 160		
15	Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu 165 170 175		
	His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg 180 185 190		
20	Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu 195 200 205		
	Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser 210 215 220		
25	Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr 225 230 235 240		
	Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn 245 250 255		
30	Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala 260 265 270		
	Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala 275 280 285		
35	Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu 290 295 300		
	Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His 305 310 315 320		
	Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro 325 330 335		
45	Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met 340 345 350		
	Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met 355 360 365		
50	Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn 370 375 380		
	Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu 385 390 395 400		
55			

EP 1 118 668 A1

Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu
 405 410 415
 5 Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser
 420 425 430
 Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn
 435 440 445
 10 Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr
 450 455 460
 Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln
 465 470 475 480
 15 Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu
 485 490 495
 Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr
 500 505 510
 20 Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val
 515 520 525
 Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu
 530 535 540
 25 Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn
 545 550 555 560
 Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala
 565 570 575
 30 Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr
 580 585 590
 His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu
 595 600 605
 35 Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala
 610 615 620
 40 Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr
 625 630 635 640
 Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr
 645 650 655
 45 Gly Ala Met Val Arg Leu Leu Glu Asp Gly
 660 665

50 <210> 9
 <211> 33
 <212> DNA
 <213> Human immunodeficiency virus

55 <400> 9
 tacggccgca agaagcgccg ccaacgccgc cgc

33

<210> 10
 <211> 11
 5 <212> PRT
 <213> Human immunodeficiency virus

 <400> 10
 Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
 10 1 5 10

 <210> 11
 <211> 42
 15 <212> DNA
 <213> Human immunodeficiency virus

 <220>
 <221> CDS
 <222> (4)..(42)
 20
 <400> 11
 atg ggc tac ggc cgc aag aag cgc cgc caa cgc cgc cgc ggc 42
 Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly
 1 5 10
 25
 <210> 12
 <211> 13
 <212> PRT
 30 <213> Human immunodeficiency virus

 <400> 12
 Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly
 1 5 10
 35

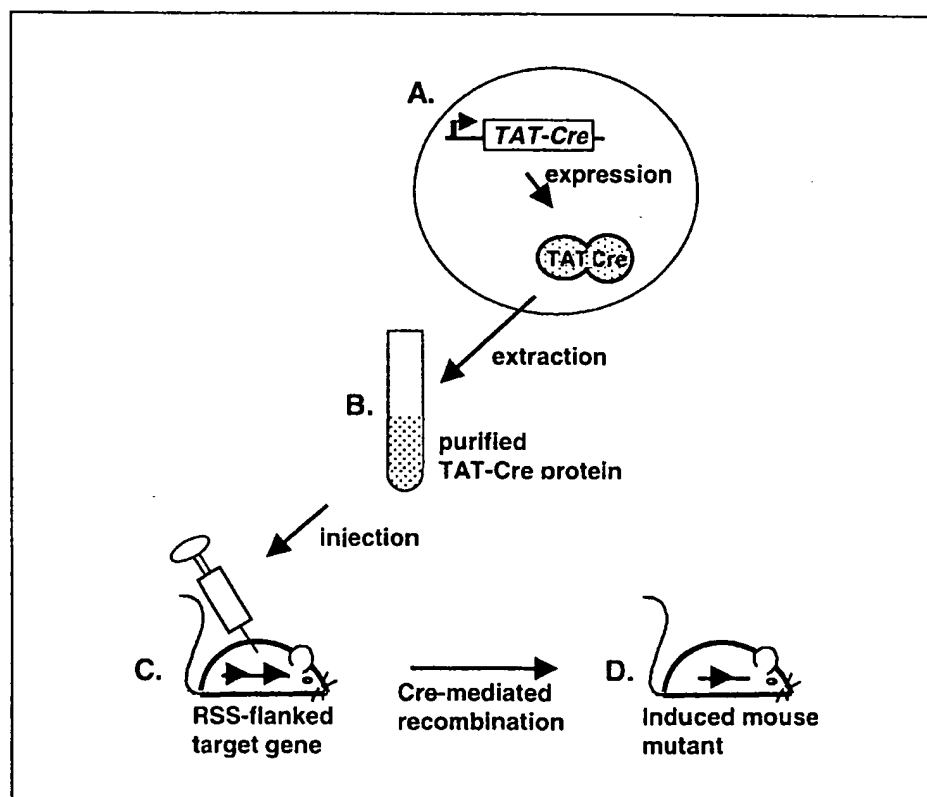
Claims

- 40 1. Use of a fusion protein comprising
- (a) a site-specific DNA recombinase domain and
- (b) a protein transduction domain
- 45 for preparing an agent for inducing target gene alterations in a living organism, wherein said living organism carries at least one or more recognition sites for said site-specific DNA recombinase integrated in its genome.
2. The use of claim 1 wherein the site-specific DNA recombinase domain is selected from a recombinase protein derived from Cre, Flp, ϕ C31 recombinase, and R recombinase and preferably is Cre having amino acids 15 to 357 of SEQ ID NO: 2 or Flpe having amino acids 15 to 437 of SEQ ID NO: 4.
- 50 3. The use of claim 1 or 2 wherein the protein transduction domain is a protein derived from the Antennapedia protein of Drosophila, from the VP22 protein of HSV or from the TAT protein of HIV, and preferably is derived from the TAT protein.
- 55 4. The use of claim 3, wherein the TAT protein comprises the amino acid sequence

YGRKKRQRRR (SEQ ID NO: 10).

- 5 5. The use of claims 1 to 4, wherein the protein transduction domain is fused to the N-terminal of the site-specific DNA recombinase domain.
6. The use of claims 1 to 5, wherein the protein transduction domain is fused to the site-specific DNA recombinase domain through a direct chemical bond or through a linker molecule.
- 10 7. The use of claim 6, wherein the linker molecule is a short peptide having 1 to 20, preferably 1 to 10 amino acid residues.
8. The use of claims 1 to 7, wherein said fusion protein further comprises additional functional sequences.
- 15 9. The use of claim 1, wherein the fusion protein has the sequence shown in SEQ ID NOs: 2, 4, 6 or 8.
10. The use of claims 1 to 8, wherein the living organism is a vertebrate, preferably a rodent or a fish.
- 20 11. A method for inducing gene alterations in a living organism which comprises administering to said living organism, a fusion protein comprising a site-specific DNA recombinase domain and a protein transduction domain as defined in claims 1 to 9, wherein said living organism carries at least one or more recognition sites for said site-specific DNA recombinase integrated in its genome.
- 25 12. A fusion protein comprising
 - (a) a site-specific DNA recombinase domain and
 - (b) a protein transduction domainprovided that when (a) is Flp then (b) is not the VP22 protein of HSV.
- 30 13. The fusion protein of claim 11 being as defined in claims 2 to 9.
14. The fusion of claim 12 or 13, wherein the protein transduction domain is derived from the TAT protein of HIV.
- 35 15. A DNA sequence coding for the fusion protein of claim 12.
16. The DNA sequence of claim 15 comprising the sequence shown in SEQ ID NOs: 9 and/or 11.
- 40 17. A vector comprising the DNA sequence of claim 15.
18. A host cell transformed with the vector of claim 17 and/or comprising the DNA of claim 15.
- 45 19. A method for producing the fusion protein of claim 11 which comprises culturing the transformed host cell of claim 17 and isolating the fusion protein.
20. An injectable composition comprising the fusion protein as defined in claims 1 to 9 or 12 to 14.

Fig. 1





European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

EP 00 10 0351

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 99 11809 A (IMP COLLEGE INNOVATIONS LTD ; CRISANTI ANDREA (GB)) 11 March 1999 (1999-03-11) * example 3 *	1-3, 5-8, 10-13, 15, 17-20	C12N15/62 C12N9/00 C12N5/10 C12N1/21 C07K14/435 C07K14/035 C07K14/16 A01K67/027 A61K38/43 A61K47/48
X	WO 99 60142 A (HENDRY JOLYON HINDSON ; MARPLES BRIAN (GB); SCOTT SIMON (GB); CANCE) 25 November 1999 (1999-11-25) * claim 9 *	1-3, 5-13, 15, 17-20	
D, X	INVITROGEN: "Voyager(TM) - The power of Translocation" EXPRESSIONS, vol. 6, no. 1, February 1999 (1999-02), page 6 XP002140132 * column 1, paragraph 7 *	13	
A	SCHWARZE S ET AL: "In vivo protein transduction: delivery of a biologically active protein into the mouse" SCIENCE., vol. 285, no. 5433, 3 September 1999 (1999-09-03), pages 1569-1572, XP002140133 --- -/--		
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			C12N C07K A01K A61K
INCOMPLETE SEARCH			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>Although claim 11 is directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		14 June 2000	Lonnoy, O
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone</p> <p>Y : particularly relevant if combined with another document of the same category</p> <p>A : technological background</p> <p>O : non-written disclosure</p> <p>P : intermediate document</p>		<p>T : theory or principle underlying the invention</p> <p>E : earlier patent document, but published on, or after the filing date</p> <p>D : document cited in the application</p> <p>L : document cited for other reasons</p> <p>& : member of the same patent family, corresponding document</p>	

EPO FORM 1503 03 82 (P04C07)



European Patent
Office

Application Number
EP 00 10 0351

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☒ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 00 10 0351

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	WO 95 00555 A (EUROP MOLECULAR BIOLOGY LAB EM ; STEWART FRANCIS (DE)) 5 January 1995 (1995-01-05) -----		
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)



European Patent
Office

**LACK OF UNITY OF INVENTION
SHEET B**

Application Number
EP 00 10 0351

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-3,5-8,10-13,15,17-20 (all partially)

Use of a fusion protein comprising a site-specific DNA recombinase domain and a protein transduction domain for preparing an agent for inducing target gene alterations in a living organism carrying at least one recognition site in its genome; said use wherein the site-specific recombinase is the Cre recombinase and said protein transduction domain is derived from Antennapedia protein of *Drosophila*; corresponding method, fusion protein, DNA sequence, vector, host cell and composition.

2. Claims: 1-3,5-13,15,17-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the Cre recombinase and said protein transduction domain is derived from VP22 of HSV, eventually as presented in SeqIdNo.6

3. Claims: 1-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the Cre recombinase and said protein transduction domain is derived from Tat of HIV, eventually as presented in SeqIdNo.2, eventually comprising the Tat-derived sequences of SeqIdNo.9, SeqIdNo.10 or SeqIdNo.11

4. Claims: 1-3,5-8,10-13,15,17-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the Flp recombinase or its modified variant Flpe, and said protein transduction domain is derived from AntP of *Drosophila*

5. Claims: 1-3,5-13,15,17-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the Flp recombinase or its modified variant Flpe, and said protein transduction domain is derived from VP22 of HSV, eventually as presented in SeqIdNo.8

6. Claims: 1-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the Flp recombinase or its modified variant Flpe, and said protein transduction domain is derived from Tat of HIV, eventually as presented in SeqIdNo.4, eventually comprising



European Patent
Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 00 10 0351

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

the Tat-derived sequences of SeqIdNo.9, SeqIdNo.10 or SeqIdNo.11

7. Claims: 1-3,5-8,10-13,15,17-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the PhiC31 recombinase, and said protein transduction domain is derived from AntP of Drosophila

8. Claims: 1-3,5-8,10-13,15,17-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the PhiC31 recombinase, and said protein transduction domain is derived from VP22 of HSV

9. Claims: 1-8,10-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the PhiC31 recombinase, and said protein transduction domain is derived from Tat of HIV, eventually comprising the Tat-derived sequences of SeqIdNo.9, SeqIdNo.10 or SeqIdNo.11

10. Claims: 1-3,5-8,10-13,15,17-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the R recombinase, and said protein transduction domain is derived from AntP of Drosophila

11. Claims: 1-3,5-8,10-13,15,17-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the R recombinase, and said protein transduction domain is derived from VP22 of HSV

12. Claims: 1-8,10-20 (all partially)

As for subject 1, but wherein said site-specific recombinase is the R recombinase, and said protein transduction domain is derived from Tat of HIV, eventually comprising the Tat-derived sequences of SeqIdNo.9, SeqIdNo.10 or SeqIdNo.11

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 00 10 0351

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

14-06-2000

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9911809	A	11-03-1999	AU	8877698 A	22-03-1999
WO 9960142	A	25-11-1999	AU	3937599 A	06-12-1999
WO 9500555	A	05-01-1995	EP	0632054 A	04-01-1995
			AT	152123 T	15-05-1997
			AU	7227994 A	17-01-1995
			CA	2166198 A	05-01-1995
			DE	69402863 D	28-05-1997
			DE	69402863 T	31-07-1997
			EP	0707599 A	24-04-1996
			ES	2101552 T	01-07-1997
			JP	2986915 B	06-12-1999
			JP	8511681 T	10-12-1996
			US	6040430 A	21-03-2000

EPO FORM P0439

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

Thank you for your request. Here are the latest results from the TARR web server.

This page was generated by the TARR system on 2005-10-18 12:48:46 ET

Serial Number: 76605863

Registration Number: (NOT AVAILABLE)

Mark



Standard Character claim: No

Current Status: Opposition period completed, a Notice of Allowance has been issued.

Date of Status: 2005-07-05

Filing Date: 2004-08-06

The Notice of Allowance Date is: 2005-07-05

Transformed into a National Application: No

Registration Date: (DATE NOT AVAILABLE)

Register: Principal

Law Office Assigned: LAW OFFICE 105

Attorney Assigned:
BELL MARLENE D Employee Location

Current Location: 700 -Intent To Use Section

Date In Location: 2005-07-05

LAST APPLICANT(S)/OWNER(S) OF RECORD

1. STACK9 SYSTEMS CORP.

Address:
STACK9 SYSTEMS CORP.
5600 Airport Blvd.

Boulder, CO 80301

United States

Legal Entity Type: Corporation

State or Country of Incorporation: Colorado

GOODS AND/OR SERVICES

International Class: 009

Computer hardware and software for providing and performing multiple functions, namely-- as a computer server for digital audio and video recording, for digital audio and video playback, for Internet communications, for managing general purpose computers, and for providing residential and business control for lighting systems, for security systems, for HVAC systems, for irrigation systems, for power management and operation systems, and for electrical appliances

First Use Date: (DATE NOT AVAILABLE)

First Use in Commerce Date: (DATE NOT AVAILABLE)

Basis: 1(b)

ADDITIONAL INFORMATION

(NOT AVAILABLE)

MADRID PROTOCOL INFORMATION

(NOT AVAILABLE)

PROSECUTION HISTORY

2005-07-05 - Notice of allowance - mailed

2005-04-12 - Published for opposition

2005-03-23 - Notice of publication

2005-01-13 - Law Office Publication Review Completed

2005-01-07 - Assigned To LIE

2004-12-26 - Approved for Pub - Principal Register (Initial exam)

2004-12-22 - Case file assigned to examining attorney

2004-08-18 - New Application Entered In Tram

CORRESPONDENCE INFORMATION

Correspondent

Donald W. Margolis (Attorney of record)

DONALD W. MARGOLIS
MARGOLIS & ASSOCIATES
PO BOX 20338
BOULDER CO 80308-3338

Phone Number: (303) 443-6200

Fax Number: (303) 443-3818
